

Amendments to the Claims

Claim 1 (Currently amended):

A method for establishing a vector packaging cell

line and sufficiently increasing the production of viral vector titer in the vector packaging cell comprising:

- 1b
- a) introducing a helper virus into a cell population to establish a packaging cell line, wherein said helper virus comprises ~~comprising a genome sequence viral structural proteins retroviral genes encoding virion structural proteins for retrovirus assembly~~ in combination with an internal ribosome entry site with linked to a selection marker and a nucleotide sequence, the presence of which enables a host cell to be capable of expressing an introduced viral components to form a viral particle so that said viral vector may be assembled into the viral particle to form an infectious viral particle; and
- increasing the vector production by inhibiting the presence of DNA methylation of helper virus in said cell eliminating cells with methylated helper virus thereby increasing virion production
- b) selecting for helper virus-containing cells from step (a) in which the helper virus DNA is not methylated by growing the cells in the presence of a selection agent thereby reducing the presence of methylated helper virus;
- c) transducing the packaging cell line with a viral vector comprising a packaging sequence and a gene of interest; and
- d) collecting viral particles.

Claim 2 (Currently amended):

The method of claim 1 wherein said step of eliminating

selecting for helper virus-containing cells in which the helper virus DNA is not methylated cells

~~with methylated helper virus~~ comprises:

positively selecting helper virus which is functional.

Claim 3 (Original): The method of claim 2 wherein said selection is by antibiotic resistance.

Claim 4 (Original): The method of claim 3 wherein said antibiotic resistance selection is accomplished via ligation of an internal ribosome entry site with a selection marker so that drug selection ensures promoter function in said helper virus.

Claim 5 (Original): The method of claim 1 wherein said viral titer achieves levels of  $1.5 \times 10^7$  cfu/ml in the presence of antibiotic resistant selection.

DL  
Claim 6 (Original): The method of claim 1 wherein said helper virus comprises at least one viral production gene operably linked to viral promoter sequence which is capable of being methylated.

Claim 7 (Original): The method of claim 6 wherein said viral promoter comprises a long terminal repeat.

Claim 8 (Currently amended): The method of claim 7 wherein said viral promoter sequence is the long terminal repeat LTR of a retrovirus.

Claim 9 (Currently amended): The method of claim 3 1 wherein said ~~selection eliminates cells with~~ growing cells in the presence of the selection agent positively selects for cells in which the methylated 5' long terminal repeat of the helper virus is not methylated-LTR.

Claim 10 (Currently amended): The method of claim 1 wherein said selecting for helper virus DNA not methylated ~~eliminating methylation~~ is accomplished by treating vector producer cells with Zeocin 5-AZA-C.

Claims 11-13 (Withdrawn)

Claim 14 (Cancelled)

Claims 15-25 (Withdrawn)

Db  
Claim 26 (Currently amended): A method for increasing the presence of viral titer produced by a vector packaging cell upon transfection with a viral vector comprising:  
decreasing the amount of inactive helper virus present in said vector packaging cell by providing for the selection of non-methylated helper virus-containing cells ~~elimination of or prevention of methylated helper virus.~~

Claim 27 (Currently amended): A method of claim 26 wherein said step of ~~decreasing~~ selecting for non-methylated inactive helper virus comprises the step of: selecting for functional ~~eliminating methylation of helper virus.~~

Claim 28 (Currently amended): The method of claim 26 wherein said step of decreasing inactive helper virus comprises the steps of:  
removing from a population of vector packaging cells, helper virus with 5' long terminal repeat methylation.

Claim 29 (Currently amended): The method of claim 28 further comprising the steps of:  
removing cells with inactivated virus by positive selection.

Claim 30 (Currently amended): The method of claim 29 wherein said removal step comprises:  
introducing an antibiotic to said cells so that cells with inactive helper virus are killed.

Db Claim 31 (Original): The method of claim 30 wherein said removal is accomplished by a helper virus with a picarnovirus internal ribosomal entry site sequence followed by an antibiotic resistance marker at the 3' end of the env sequence of said helper virus.

Claim 32 (Original): The method of claim 31 wherein said antibiotic resistance selection marker is Zeocin.

Claim 33 (Currently amended): The method of claim 27 ~~further comprising wherein said~~  
~~inhibiting of methylation is accomplished by a step selected from the group consisting of:~~  
treating of vector producer cells with 5-AZA-C.

Claim 34 (Withdrawn)

Claim 35 (Currently amended): The method of claim 27 wherein said selecting for ~~inhibiting of non-methylated helper virus methylation~~ is accomplished by a step selected from the group consisting of:

ligation of an internal ribosome entry site with a selection marker so that drug selection would ensure promoter function.

DK